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Two Different Species of Murein Transglycosylase in *Escherichia coli*

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We demonstrated that *Escherichia coli* murein transglycosylase exists in two forms. After mechanical disruption of the cells, one form was found in the soluble fraction and the other, in the cell envelope. The two enzymes differed with respect to molecular weight, isoelectric point, solubility in aqueous buffers, and to some extent in their requirements for maximal catalytic activity. The molecular weight of the membrane-bound transglycosylase (35,000) was half that of the soluble enzyme. Whether the high-molecular-weight soluble protein is a precursor of the membrane-bound enzyme species remains to be elucidated.

A set of hydrolytic enzymes is engaged in the metabolism of the murein sacculus (25) of bacteria. Since the sacculus maintains bacterial cell shape, murein hydrolases are assumed to be directly involved in bacterial morphogenesis (2, 3, 14, 15).

The set of different murein hydrolases in *Escherichia coli* includes transglycosylase activity which degrades the sacculus completely into low-molecular-weight subunits. The disaccharide peptides liberated upon hydrolysis of the glycan chains of the sacculus have internal 1,6-anhydro linkages in the muramic acid residues (7).

In our attempts to elucidate the biological function of transglycosylase, we searched for the most abundant hydrolase activity in *E. coli* mutants with altered activity. All mutants obtained so far showed, however, a high residual activity (E. W. Goodell and U. Schwarz, unpublished data). The data presented here may explain this: murein transglycosylase exists in two distinct molecular species, a soluble and a membrane-bound protein.

MATERIALS AND METHODS

Bacterial strain and growth conditions. The strain used in this investigation was *E. coli* PA3092 (M. Ricard, Ph.D. thesis, Faculté des Sciences, Paris, France). Bacteria were grown with vigorous aeration at 37°C in nutrient broth medium (Difco Laboratories), supplemented (18) with NaCl (0.5%), lysine (20 μg/ml), thymine (50 μg/ml), and DL-α,ε-diaminopimelic acid (Dpm; 4 μg/ml). At a density of approximately 10^8 to 5×10^8 cells per ml, the cultures were rapidly chilled by addition of ice and harvested (Sharples continuous-flow rotor). After addition of a small volume of saline, the cells were stored at −70°C.

Preparation of crude transglycosylase extracts (all steps at 0 to 4°C). Soluble transglycosylase was isolated essentially as described (7; H. Mett, Ph.D. thesis, Universität Tübingen, Tubingen, West Germany) from a cell extract obtained by disruption of the cells by shaking with glass beads and sedimentation of the remaining cell envelopes.

Routinely, the envelopes obtained from 500 g of frozen cells were used for preparation of the membrane-bound transglycosylase. The pellet, obtained after separation of cytoplasmic material, was washed once with 750 ml of 10 mM Tris-maleate (pH 6.5)-10 mM EDTA and once in 750 ml of 10 mM Tris-maleate (pH 6.5). In each case, the envelopes were sedimented for 1 h at 35,000 × *g*. The final pellet was used as a purified envelope preparation. This material was solubilized at a concentration of 0.1 g (wet weight) of envelopes per ml in a solubilization buffer containing 10 mM Tris-maleate (pH 6.5), 1 M KCl, and 1% Triton X-100 (technical grade, Serva, Heidelberg, West Germany). After being stirred for 90 min in the cold, the suspension was clarified (1 h at 35,000 × *g*), and the resulting pellet was washed once with a small amount of solubilization buffer. The combined supernatants were dialyzed against solubilization buffer lacking KCl (three times, 5 liters), and the turbid dialysate was cleared by centrifugation (1 h at 35,000 × *g*). The supernatant was used as a crude extract of membrane-bound transglycosylase.

Analytical methods. Sodium dodecyl sulfate slab gel electrophoresis was performed by a slightly modified (Mett, Ph.D. thesis) version of Lugtenberg's method (11). Gels were stained as described by Weber and Osborn (24). Protein was determined by the method of Lowry et al. (10), with bovine serum albumin as a standard. Isoelectric focusing was performed either in polyacrylamide gels (23) or in Ultrodex gels (as described by the manufacturer of the gel). Radioactivity was routinely determined in 0.1- to 0.2-ml neutralized aqueous solutions with the use of 2 ml of a Triton-toluene-PPO (2,5-diphenyloxazole) scintillation cocktail (4). Radioactivity on paper chromatograms was located by routine procedure (7).

We tested the enzymatic activity of the transglycosylases using the natural substrate (12) *E. coli* murein sacculi, which were radioactively labeled in the Dpm (4). Low-molecular-weight products released by en-

zyme digestion were separated from high-molecular-weight murein substrate by trichloroacetic acid precipitation (4). In standard assays, 5 μg of ^{3}H-labeled sacculi (specific activity, 2×10^3 to 4×10^3 cpm/μg of murein) was incubated in a total volume of 100 μl of buffer (10 mM Tris-maleate, pH 6.0, 10 mM $MgSO_4$, and 0.2% Triton) for 15 to 60 min at 37°C. Specific activity of transglycosylase was expressed as picomoles of Dpm rendered trichloroacetic acid soluble per microgram of protein per hour of incubation at 37°C. One enzyme unit is the amount of protein leading to solubilization of 1 pmol of Dpm in 1 h at 37°C.

For examination of the enzyme specificity of the murein hydrolase preparation, the whole murein digestion mixture was spotted on paper (Schleicher & Schuell SS 2040b) and chromatographed descending for 18 to 30 h in the upper phase of a solvent (7) composed of *n*-butanol–acetic acid–water (4:1:5). Murein concentrations were determined by measurement of the Dpm content (26) of acid hydrolysates (6 N HCl, 14 h, 105°C, in sealed tubes under N_2). Molarities were expressed by using a murein-subunit molecular weight of 918.

2,6-[4(*n*)-^{3}H]Diaminopimelic acid (specific activity, 35 Ci/mmol), was obtained from Services des Molécules Marquées, Gif-sur-Yvette, France.

If not stated otherwise, chemicals were from Merck (Darmstadt, West Germany) and were all of the highest purity available. All solutions expressed as percentages are weight per volume.

RESULTS

Purification of transglycosylase (all steps at 0 to 4°C). The purification of the soluble (cytoplasmic) fraction of the transglycosylase was essentially performed as described by Höltje (7). After mechanically opening the cells (see Materials and Methods), the supernatant was treated with 50% saturated ammonium sulfate. The resulting precipitate was, after resuspension and dialysis, chromatographed on Bio-Gel A-1.5m in 10 mM phosphate (pH 6.9)–20% saturated ammonium sulfate. Enzyme activity eluted in fractions corresponding to low-molecular-weight compounds, indicating a possible hydrophobic interaction of the protein with the gel matrix. This assumption was confirmed by experiments in which crude enzyme fractions were loaded on agarose columns equilibrated with buffers containing 40% saturated ammonium sulfate. In these cases the enzyme was completely bound by the gel, and elution could be achieved with a decreasing gradient of ammonium sulfate. Enzyme activity was eluted at approximately 30% saturated ammonium sulfate (Fig. 1). This method ("salting out chromatography") was described by van der Haar (22).

Fractions from the agarose column containing transglycosylase activity were pooled and dialyzed against 10 mM Tris-hydrochloride, pH 8.0. Further purification was achieved by ion-exchange chromatography on DE 52 cellulose (Whatman). The adsorbed enzyme was eluted with an NaCl gradient at approximately 50 mM NaCl. This material was dialyzed against 10 mM phosphate buffer (pH 6.9) and then loaded onto a small column of hydroxylapatite (Bio-Gel HT, Bio-Rad Laboratories). Desorption of enzyme activity was achieved with a linear phosphate gradient, active fractions eluting at approximately 80 mM potassium phosphate. After this step, the enzyme was chromatographed on a column of agarose (Bio-Gel A-0.5m) or Sephadex G-150 equilibrated with high concentrations of salt (10 mM KPO_4–0.5 M KCl, pH 6.9). Under these conditions the enzyme eluted at a position

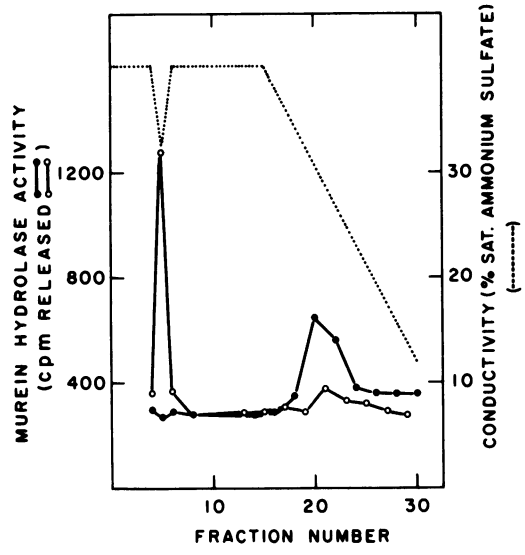

FIG. 1. *Influence of ammonium sulfate concentration on the behavior of crude cytoplasmic transglycosylase on agarose columns. A column of agarose (Bio-Gel A-1.5m; gel bed, 1.6 by 8.5 cm) was equilibrated with 40% saturated ammonium sulfate in 10 mM potassium phosphate buffer, pH 6.9, at a flow rate of 20 ml/h. Crude cytoplasmic transglycosylase was dialyzed against 30% saturated (●) and 10% saturated (○) ammonium sulfate in 10 mM potassium phosphate buffer, pH 6.9; insoluble material was removed by centrifugation (15 min at 15,000 × g), and 5-ml amounts of the supernatants were loaded on the column in two separate experiments. The columns were eluted with 40 ml of starting buffer; then a linear decreasing gradient of ammonium sulfate was applied (50 ml of 40% and 50 ml of 10% saturated ammonium sulfate in 10 mM potassium phosphate, pH 6.9); elution velocity was 20 ml/h, and fractions of 5 ml were collected. The conductivity was continuously monitored with a flow cell (0.3 ml) and a conductometer (WTW, Weilheim, West Germany) connected to a recorder. Murein hydrolase activity was tested by using 5 μl of effluent in the standard assay. The assays were incubated for 1 h at 37°C.*

equivalent to a Stokes radius of 3.4 nm or a molecular weight of 64,000 (Fig. 2A).

The envelope-bound fraction of transglycosylase was purified in a different manner as a result of the enzyme's requirement of Triton X-100 for solubility. Because of the difficulty in adsorbing the crude enzyme onto conventional ion-exchange resins, two procedures were employed as the first step of purification. In the first procedure, the enzyme activity was adsorbed onto pretreated chitin; the chitin was purified from crab shells (Sigma Chemical Co.) by a procedure (21) including solubilization of the chitin in concentrated HCl and reprecipitating the dissolved *N*-acetylglucosamine polymers in cold water. The adsorption of the enzyme onto this HCl-chitin was performed in a batch procedure; desorption was achieved by washing the chitin with 0.3 to 0.5 M KCl in standard buffer (10 mM Tris-maleate [pH 6.5] and 1% Triton). In the second procedure the crude enzyme preparation was repeatedly passed through columns of blue agarose gel (Blue Sepharose CL-6B, Pharmacia, Freiburg, West Germany). The columns, for unknown reasons, retained only about 20% of the enzyme per run. After repeated chromatography of the enzyme solution, most of the enzyme activity could be finally recovered. After this first step, conventional chromatographic techniques could be applied, again using Triton-containing buffers. The next two steps consisted of ion-exchange chromatography on a column of carboxymethyl cellulose (CM-32, Whatman) at pH 6.5 (10 mM Tris-maleate, 1% Triton; enzymatic activity eluted in a linear KCl gradient at about 0.1 M KCl) followed by a column of hydroxylapatite (Bio-Gel HT) in potassium phosphate buffer, pH 6.9, containing 1% Triton. The enzyme was eluted from hydroxylapatite with a phosphate gradient at about 80 mM phosphate. Additional purification was achieved by chromatography over Sephadex G-150 sf (Pharmacia) in the presence of 0.5 M KCl and 0.1% Triton. Enzymatically active material eluted in the void volume (Fig. 2B). The enzyme was purified in the final step by centrifugation on sucrose density gradients (7 to 36%) (16). Under the conditions used, the enzyme banded in the center of the gradient at about 22% sucrose as determined by refractometry (Fig. 3). Data on the purification of both enzyme fractions are summarized in Table 1. Both enzyme fractions were pure as judged by sodium dodecyl sulfate gel electrophoresis (Fig. 5).

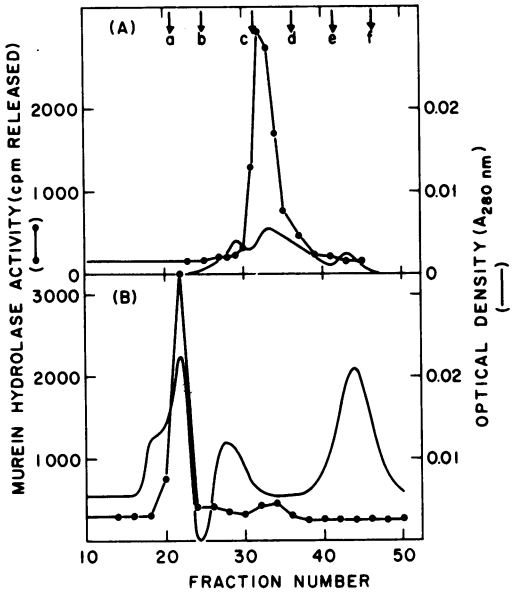

FIG. 2. *Purification of cytoplasmic (A) and membrane-derived (B) transglycosylase fractions over a Sephadex column. The column (Sephadex G-150 sf; 2.5 by 73.8 cm) was run upwards with a flow rate of 6.1 ml/36 min per fraction. The calibration run (1 mg each of Blue Dextran 200 [a], guinea pig immunoglobulin G [b], bovine serum albumin [c], chicken ovalbumin [d], bovine chymotrypsinogen A [e], and horse cytochrome c [f]) and the run for purification of the soluble enzyme fraction were performed with 10 mM potassium phosphate (pH 6.9)–500 mM KCl. For purification of membrane-derived transglycosylase, the agarose column was equilibrated and run in 10 mM Tris-maleate [pH 6.5]–0.1% Triton–500 mM KCl. Enzyme activity was determined using 3 μl (soluble fraction) or 5 μl (membrane-derived fraction) of eluent under standard conditions. UV absorbance was monitored at 280 nm with an ISCO UA5 monitor.*

Enzyme activity of the two fractions of transglycosylase. Upon digestion of murein sacculi, both enzyme fractions released as major fragments the muropeptides X/X′ and the corresponding dimer (7). When impure enzyme preparations were used, the dimer peak disappeared as a result of the action of contaminant endopeptidases (8). An additional reaction product found on the chromatograms between the origin and the position of the muropeptide dimers was not analyzed further. It probably contained oligomeric digestion products which are no longer cleavable by transglycosylase (the spot did not disappear upon prolonged digestion with the enzyme).

Other potential substrates for the enzymes which were tested by us (e.g., murein sacculi prepared from *Bacillus megaterium* or *Micrococcus luteus* and polymer secreted by *M. luteus* during incubation in penicillin-containing medium [13]) were completely resistant to digestion by the membrane-derived enzyme fraction, the only one so far examined.

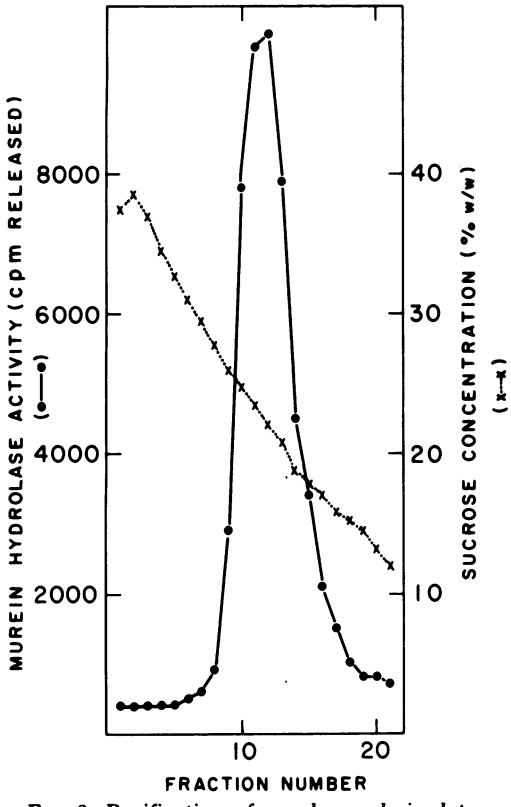

FIG. 3. *Purification of membrane-derived transglycosylase in a sucrose gradient. Gradients contained 5 ml of 7 to 36% sucrose in 10 mM Tris-maleate [pH 6.5]–300 mM KCl–0.05% Triton; 0.25-ml samples were overlayered. Centrifugation was for 45 h in a Beckman Spinco L2-65 centrifuge in an SW65 rotor at 55,000 rpm (220,000 × g). The gradients were harvested from the bottom of the tubes, and 260-μl fractions were collected. Sucrose concentration was read in a Zeiss refractometer at room temperature without corrections. Murein hydrolase activity (●) was determined under standard conditions using 1 μl of each fraction.*

Enzymatic parameters. Both enzyme fractions were totally inactive in the absence of Triton, but whereas the membrane-derived fraction could only be maintained in solution in the presence of a detergent, the soluble enzyme remained in solution in detergent-free buffer. Upon removal of the detergent on an HT column, the membrane enzyme aggregated and was pelleted by high-speed centrifugation (45 min at 200,000 × *g*). Charge shift electrophoresis (6) was performed in a flat bed of Sephadex G-25 to test whether the enzyme proteins bind substantial amounts of detergent. The migration of both enzyme fractions was influenced by anionic and cationic detergents, indicating an interaction of enzyme protein with the detergents (data not shown).

For determination of the Michaelis constants (9), incubation mixtures containing various concentrations of murein sacculi were assayed in the standard way. After 15 or 30 min, the incubations were stopped, and the amount of solubilized murein was determined (see Materials and Methods). For the two enzyme preparations, the values listed in Table 2 were obtained.

The pH optima of the two enzymes were obtained by using as incubation buffers 10-fold concentrated Tris-maleate buffers or sodium acetate buffers (100 mM each) containing Mg^{2+} (100 mM) and Triton (2%); the pH was adjusted with KOH. The pH optima shown in Fig. 4A were fairly similar for the two enzyme species. From the studies of Höltje (7), the properties of the transglycosylase of a different *E. coli* strain are known. That enzyme exhibited an unusual preference for acetate, being most active in the presence of 0.4 M sodium acetate, pH 4.5; under these conditions, Mg^{2+} had no effect on the enzymatic activity. For comparison with those observations, our enzymes were tested under similar conditions. The results (Fig. 4B) show that the soluble enzyme behaved as did the enzyme isolated previously (7) from the W7 strain. The activity of the membrane enzyme was slightly stimulated by acetate, when assayed in buffer free from Mg^{2+}. However, marked dependence on magnesium was found: activity was optimal at 5 to 20 mM $MgCl_2$, and the activity at 10 mM Mg^{2+} and 10 mM acetate was twice as high as the activity in 200 mM acetate without Mg^{2+}. The soluble enzyme was not specifically influenced by magnesium (data not shown).

Molecular weights of the two species of transglycosylase. The native molecular weight of the soluble transglycosylase was determined on a column of Sephadex G-150, and the native molecular weight of the membrane-derived enzyme was determined on an agarose column (Bio-Gel A-1.5m). As can be seen in Fig. 2, the native molecular weight of the soluble enzyme fraction was slightly lower than the molecular weight of bovine serum albumin; it was estimated to be 64,000. For the membrane-derived enzyme fraction, the situation was somewhat more complicated. Enzymatic activity was eluted from the agarose column in the presence of Triton immediately ahead of native guinea pig immunoglobulin G (apparent separation coefficients K_{av}, as defined by Pharmacia, were 0.51 for the transglycosylase and 0.52 for immunoglobulin G; molecular weight, about 150,000). However, a great contribution to this apparently large Stokes' radius came from en-

TABLE 1. *Purification of the two transglycosylase species*[a]

Fraction	Purification step	Protein		Enzyme activity (pmol of Dpm released)	
		mg/ml	Total amt (mg)	Sp act	Total activity
Cytoplasmic (from 750 g of frozen cells)	Crude extract	29.2	41,500	4.4	174
	Redissolved material after ammonium sulfate precipitation	31.3	19,200	14.7	251
	Agarose eluate	0.492	517	491	251
	DEAE eluate	0.091	41	3,160	131
	Hydroxylapatite	0.207	10	4,580	45.8
	Sephadex G-150	0.081	0.5	88,300	41.5
Membrane derived (from 525 g of frozen cells)	Crude extract	3.75	8,370	152	1,270
	Blue Sepharose (combined pool of three successive runs)	0.715	1,200	327	392
	Carboxymethyl cellulose	0.289	196	1,360	267
	Hydroxylapatite	0.535	88	1,420	125
	Concentration over carboxymethyl cellulose	1.52	25	6,920	136
	Sephadex G-150	0.032	1.2	54,500	65.4
	Sucrose gradient	0.052	0.4	150,000	60.5

[a] The purification steps are described in the text. Enzyme activity was determined by use of pH 4.5 buffer (cytoplasmic fraction) or pH 6 buffer (membrane-derived fraction). The assays were incubated for 15 to 30 min at 37°C; for further details, see Materials and Methods.

TABLE 2. *Maximal velocities and Michaelis constants of the two transglycosylase preparations*[a]

Enzyme fraction	K_m (μM)	V_{max} (μmol/liter·min)
Soluble	64	0.59
Membrane derived	28	1.24

[a] Assays contained, in a final volume of 100 μl, up to 80 μl of Dpm-labeled murein sacculi (0.5 mg/ml, 10^6 cpm/ml). Incubation was performed at 25°C for 30 min. Soluble enzyme (5 μl containing 10,500 U; specific activity, 18,800) was incubated at pH 4.5 in 10 mM sodium acetate buffer containing 0.2% Triton and 10 mM $MgSO_4$. Membrane-derived enzyme (2 μl containing 15,600 U; specific activity, 150,000) was incubated at pH 6.0 in 10 mM Tris-maleate containing 0.2% Triton *X*-100 and 10 mM $MgSO_4$.

zyme-associated Triton (see above). When the amount of Triton bound by the enzyme protein was determined by coelution of ^{3}H-labeled Triton and enzyme on an HT column (5), a ratio of approximately 4 μg of bound Triton per μg of protein was determined. Thus, in rough estimate, only one-fifth of the weight of the Triton-enzyme complex was enzyme protein. Under this assumption, the molecular weight of the enzyme protein could be estimated to be in the range of 30,000. In this case also the generalized rule seems to be true (17) that one Triton micelle consisting of roughly 150 molecules of detergent is bound to one molecule of a membrane protein.

In the denatured state, both enzyme preparations were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (see Materials and Methods); Triton was removed before electrophoresis by precipitation of the protein with 90% acetone. In Fig. 5 the stained gels of both enzyme fractions are shown. The estimated molecular weights were 65,000 for the soluble enzyme and 35,000 for the membrane-derived fraction.

Isoelectric points. Isoelectric focusing was performed in the presence of Triton as described in Materials and Methods to determine the isoelectric points of the enzymes. The transglycosylase activity could easily be detected after elution from Ultrodex gel (LKB), but hardly any activity was recovered after focusing in polyacrylamide gels. The different isoelectric points of the two enzyme fractions are shown in Fig. 6; it should be mentioned that, in this gel focusing system, we could never reach a final stability in the pH gradient. For this reason, the observed pI values of 7.0 for the membrane enzyme and of 5.2 for the soluble enzyme can only be taken as apparent figures.

DISCUSSION

Our experiments indicate the existence of two distinct molecular species of murein transglycosylase. This is another example of the occurrence

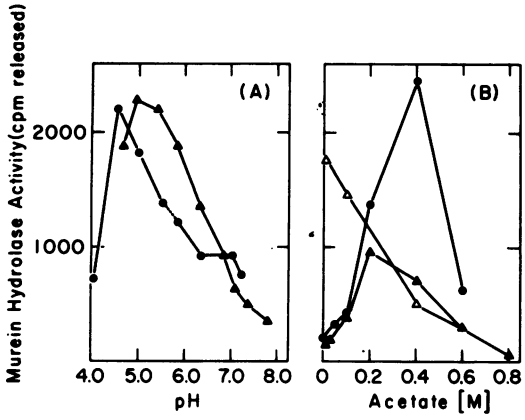

FIG. 4. *Dependence of transglycosylase activity on the pH (A) and on acetate concentration (B). (A) The cytoplasmic fraction (●) contained 50 μg of protein per ml with a specific activity of 22,200; 3 μl of this solution was incubated at 37°C for 30 min. The buffers used were acetate-NaOH from pH 4 to 5.5 and Tris-maleate from pH 5.5 to 7.5. The membrane-derived enzyme (▲) contained 6.4 μg of protein per ml with a specific activity of 65,900; 2 μl was incubated for 1 h at 37°C using Tris-maleate buffer over the pH range illustrated. For both assay series, 5 μg of substrate containing 10^4 cpm of [^{3}H]Dpm was used. The final Triton concentration was adjusted to 0.2%. The pH values were determined at room temperature using 10-fold assay volumes. (B) Cytoplasmic enzyme (3 μl; 31 μg of protein per ml; specific activity, 18,800) was incubated for 1 h at 37°C (●) in buffers of increasing acetate concentration; the pH had been adjusted to 4.5 with NaOH. Membrane-derived enzyme (see A) was incubated under identical conditions in the absence (▲) or presence (△) of 10 mM $MgSO_4$.*

of multiple enzyme species having identical specificity among the complex set of murein hydrolases in *E. coli* (8, 20). The two transglycosylase species had some properties in common but differed in others. Both proteins appeared to be rather hydrophobic, as shown by their ability to interact strongly with detergents (6, 16, 17). The soluble enzyme fraction, in contrast to the membrane-derived species, remained soluble even in the absence of detergent. From sodium dodecylsulfate-gel electrophoresis (Fig. 5) and from gel chromatography (Fig. 2) we obtained molecular weights of 65,000 and 35,000 for the cytoplasmic and the membrane-derived fractions, respectively.

In addition, we should mention that the substrate specificity, at least of the membrane-derived enzyme fraction (which is the only one so far tested), was very strict. This enzyme only cleaved *E. coli* murein sacculi and oligomeric murein subunits. *M. luteus* soluble murein polymer (13) and sacculi prepared from *M. luteus*

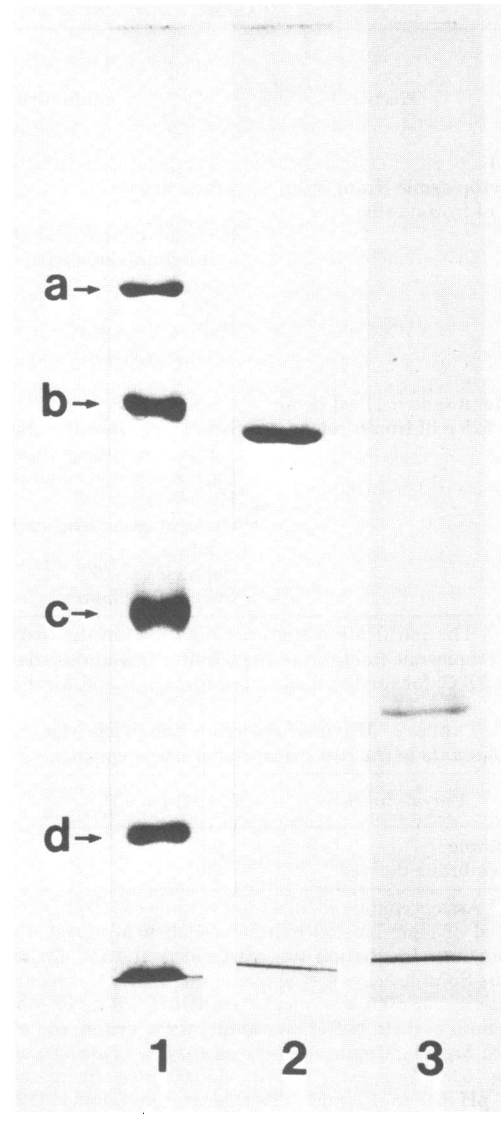

FIG. 5. *Molecular weights of the different transglycosylase species, determined by sodium dodecyl sulfate-polyacrylamide slab gel electrophoresis. Preparation and development of the gel (10% acrylamide) are described in Materials and Methods. Slot 1 contained the following calibration proteins (Pharmacia); 2 μg each: (a) phosphorylase b (94,000 daltons), (b) bovine serum albumin (68,000 daltons), (c) ovalbumin (45,000 daltons), and (d) carbonic anhydrase (30,000 daltons). Slot 2 contained soluble transglycosylase (prepared as described in the text; 4.6 μg of protein; specific activity, 124,000). Slot 3 contained membrane-bound transglycosylase (prepared according to a modified scheme including affinity chromatography on polyuridylate-Sepharose [W. Keck, to be published]; 1 μg of protein; specific activity, 153,000).*

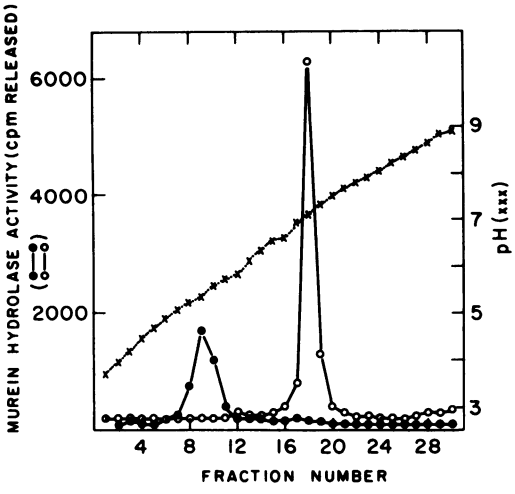

FIG. 6. *Isoelectric focusing of transglycosylase preparations. The focusing was performed on an LKB Multiphore apparatus at constant voltage (12 h at 200 V, then 2 h at 400 V, and 8 h at 600 V). As a carrier, 4% Ultrodex (LKB), 0.2% Triton X-100, and 2% Servalyte, pH 2 to 11 (Serva, Heidelberg, West Germany) were used. After evaporation of 40% of the initial weight, the plate was transferred to a thermostated support (2°C), and the samples were loaded close to the anode (fractions 3 to 5). After focusing, the gel was divided into 30 fractions, which were scraped into buffer-containing vials (0.5 ml of 10 mM Tris-maleate [pH 6.5] with 1% Triton) and shaken overnight at 4°C. After sedimentation of the gel by centrifugation, samples of the supernatant were tested for enzyme activity under standard conditions with murein sacculi as a substrate. The membrane-derived enzyme fraction (○) contained 15 μg of protein (specific activity, 30,000). The cytoplasmic enzyme fraction (●) contained 120 μg of protein (specific activity, 1,910).*

or *B. megaterium* were not substrates for the enzyme. The *B. megaterium* sacculi differ from *E. coli* sacculi in that they contain wall-bound teichoic acid instead of covalently bound lipoprotein (1).

One might speculate that the high-molecular-weight soluble enzyme species is a precursor of the low-molecular-weight membrane-bound enzyme. However, there is no good support for such a speculation. Antibodies raised against the soluble enzyme protein did not show cross-reaction with the membrane-bound enzyme fraction in double-diffusion tests. Furthermore, preliminary peptide mapping showed a marked difference in the peptide patterns obtained from the two enzymes (W. Keck, unpublished data).

The final understanding of the biological function of the two enzymes depends on the isolation of mutants with defective transglycosylases. All our attempts to isolate such mutants, however, yielded only derivatives with high residual activity. This is now explained by our demonstration of the presence of two different transglycosylase species. With the actual information in hand, it is hard to assign a specific role to the soluble enzyme, which is probably cytoplasmic, since it could not be liberated by osmotic shock (J.-V. Höltje, unpublished data). This enzyme, then, has no easy access to the high-molecular-weight sacculus as a substrate. The membrane-bound enzyme, however, is in close topological contact with murein and may catalyze intramolecular rearrangements within the sacculus during cell enlargement and cell division (19).

ACKNOWLEDGMENTS

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